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THERMAL INJURY OF THE SKIN

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OBJECTIVE

To demonstrate that following tissue injury (thermal, traumatic, radiation, chemical) there develops degradation products of tissue breakdown which can be toxic and lethal to the host. To further demonstrate that these "toxins" act as foreign bodies in the host and stimulate the production of autoantibodies.

Toward this objective the following has been accomplished up to the current period. A "toxin" has been isolated directly from diffusates of burned skin of rats *in vivo* circumventing the circulation. This "toxin" is fatal to mice and rats in acute or chronic experiments. Injection of this "toxin" plus Freund's adjuvant into rabbits produces precipitins to the "toxin" and hemoagglutinins and "hemolysins" to red cells of acutely burned rats. In preliminary experiments in mice, this rat-"toxin" rabbit anti-sera, when incubated with or injected after rat "toxin" detoxifies appreciably the action of the "toxin."

The demonstration of a "toxin" followed by autoimmunization likewise was detected by serologic means in the *blood* of burned rats and mice. Serologically the naturally occurring and the artificially produced "toxin-antitoxin" were related.

The human studies support the animal work. Thus far it has been shown that the serum of burned human subjects is cytotoxic to HeLa cells, cytolytic to injured red cells, and that the sera of healed burned humans have an enhanced capacity to neutralize the cytotoxic effect on HeLa cells *in vitro*, and clinical evidence suggests a similar *in vivo* effect. The latter effect was associated with clinical improvement.

From the results here reported and from previous experimental evidence from this laboratory, it is postulated that following injury, tissue destruction follows as a result of the injury proper. This process in turn releases or activates many enzyme systems that continue the further degradation of the tissue. These degradation products enter the blood stream and can be toxic or lethal to the host. They also can act as antigens stimulating autoimmunization in the host.

It has been shown in animals and humans by serologic and tissue culture methods that many types of injury (thermal, traumatic, radiation, or chemical) produce toxic and lethal degradation products which call forth anti-bodies which are similar but not identical.

The time of detection of cytotoxic and cytolytic agents in the blood following burning or trauma differed in these studies. One or the other of the "toxins" appear at critical times shortly after the injury and remain for varying periods dependent upon the extent of the injury.

Clinical combined with laboratory evidence suggests that sera of healed burned individuals may act as an 'antitoxin' when injected into acutely and severely burned or injured individuals. This form of therapy was first recommended by Rosenthal in 1937 and has recently gained support from Russian and Czechosolvakian sources.

The detection of "antitoxins" in the blood of burned individuals here reported was not until 40 days after the injury when healing was well on its way. It is likely that antibodies appear sooner but in the presence of an open wound with degradation products continually forming, neutralization of antibodies present in the blood probably occurred and thus they escaped detection. It is of interest in this regard that in severe burns the catabolic process is known to continue for long periods of time (30 days or more) as demonstrated by a negative nitrogen, phosphorus and potassium balance.

"Antitoxins" and/or "antibodies" ("anticytotoxins, "hemolysins," and precipitins) were detected in healed burned donors within five years after burning. The fact that these properties were found in convalescent or healed burn sera and to an extent not found in normal sera and were contained almost entirely in the gamma globulin fraction ("anticytotoxins") speaks for true antibody formation.

It is proposed that amelioration of the basic injury caused by the "burn toxins" will be necessary before the host can cope efficiently with bacteria or other noxious agents that invade it. On the basis of presently available data, it is believed that one method worthy of further investigation to effect such an amelioration is by the use of healed burned donor sera of known <u>in vitro</u> antitoxic potency.

I. CURRENT STUDIES

1. NEW OR IMPROVED SEROLOGIC PROCEDURES

A. Hemolysins (New Procedure)

In previous studies it was demonstrated that the red blood cells of an acutely burned patient would at certain critical times be hemolyzed by autologous or homologous acute burn sera and/or by the sera of a healed burned donor in the presence of guinea pig complement (table I and table II). Since the test depended upon obtaining red cells from acutely burned individuals at certain time intervals after burning (72 hours to 6 days), it was obvious that this was not a practical testing procedure. Many attempts were made to design a hemolysin test using heated (47 to 63°C.) normal human red blood cells of all blood types, but mainly Type O since it contained no interfering agglutinogens. Direct and indirect Coombs tests were also tried. However, these trials were not successful in demonstrating differences between normal and acute sera.

Animal erythrocytes were used such as guinea pig and rabbit, but they too failed to demonstrate any differences in hemolysin or hemoagglutination tests. It is well known that the serum of most individuals contain heterophilic antibodies which will react with sheep cells. These antibodies are usually of the Forssman type. In certain diseases Forssman antibody titre is increased and in Infectious Mononucleosis and occasionally in other conditions an antibody different from the Forssman type is found. These findings suggest that sheep cells used in a homolysin test might be capable of reacting with a hemolytic component in "burn" serum. Many modifications of the standard sheep cell hemolysin tests were tried using

different amounts of guinea pig complement, incubating RBC plus serum before adding complement, varying the concentration of the RBC suspension, etc., etc. (see table III), but repeatable results were not obtained that would differentiate the normal sera from the acutely burned patient sera.

It appeared however that the age of the serum played an important role in this differentiation, i.e., normal sera with ageing (stored at 4°C. or room temperature) gradually lost its ability to hemolyze sheep red cells, first without the presence of guinea pig complement and later in the presence of guinea pig complement.

It was reasoned therefore that if one could augment this ageing process, one might expedite the test and make it practical. To this end, a series of experiments were set up as follows: (1) determine the time necessary for normal sera to lose its hemolytic properties (with and without complement) (2) determine if heat (56° for 30 min., to 63° for 5 min., etc.) would inactivate the hemolytic effect (with and without complement).

Storage at icebox temperature (+4°C.) and deep freeze temperatures (-20°C.) were also compared.

The sera of acutely burned individuals was run concomitantly when available.

The actual method used for performing the test is as follows: Serial twofold dilutions of the serum are made with 0.9 percent saline starting with a 1:2 dilution. Each tube contains 0.25 cc of diluted serum plus 0.1 cc of a 2 percent suspension of sheep cells that has been washed three times. These tubes are incubated at 37°C. for one hour and read for hemolysis of the sheep cells. Then two drops or 0.1 cc of a 1:10 dilution of guinea pig complement is added (2 units).

0.5 cc of saline is also added at this time to all tubes. The tubes are shaken well and incubated for another hour at 37°C. The degree of hemolysis and final titre is recorded after this incubation.

The above method has proven to be the most sensitive and easily read of all the tests listed in table III.

Results

Normal Sera

Table IV presents the hemolysin data on control normal sera. These controls consisted of serum taken from 41 individual blood donors from two different blood banks; 2 pools of 20 additional donors; 11 sera from medical students and 4 immune donors who had recovered from burns in the past. A total of 76 sera were tested at different time intervals.

Some of these sera were stored as separated serum at 4°C. Others were stored on the clot at 4°C. or at room temperature and separated at the time of testing. The results have been combined for this presentation.

The data shows that bloods tested from 1-7 days from the time of drawing had sheep cell hemolysins in 100 percent of the non-inactivated specimens with or without the addition of complement. From 30 to 73 days, 80 to 85 percent of the specimens became negative without or with complement respectively. Inactivation at 56°C. for half an hour destroyed this hemolysin except in 2 percent of the fresh sera. However, when complement is added to inactivated sera that were 1-7 days old hemolysis reappeared in 73 percent of the cases. Sera that were 30-73 days old all failed to hemolyze sheep RBC (with and without complement).

Inactivation of the control sera at 60°C. for 15 minutes destroyed the sheep cell hemolysin and it could not be restored by the addition of complement. This was true in 100 percent of the controls.

Sera of Burned Individuals

The effect of ageing and temperature was carried out on four patients who were severely burned (45 to 65%, 2nd and 3rd degree) and all eventually died. The sera were collected from a few minutes to four hours after death. In one instance both a premortem and postmortem specimen was available (both reacted similarly).

From table V it can be seen that with ageing up to 150 days, hemolysins were present in all those tested; (with and without complement). For the normal sera, 85 and 100% were negative (without and with complement) up to 73 days of storage. After heating at 60°C. for 15 minutes, the two specimens tested retained their hemolytic properties from 1 to 14 days, but 60 to 66% remained positive after storage at 4°C. for periods up to 150 days. In the control sera all were negative at the various time intervals. Burn sera heated at 63°C. for 5 minutes lost its hemolytic properties with and without complement.

Thus it would appear that differences between the normal and burn sera were evident in all three methods used, i.e., storage, heating at 56 to 60°C.

The question of loss of hemolytic properties of sera when frozen for long periods of time was explored. Normal sera frozen for 199 to 660 days were compared with burn sera stored for 360 to 455 days (table VI). Of 15 "burn" sera in seven patients, all contained hemolysins when complement was added to non-inactivated serum. After inactivation for half an hour at 56°C. and adding complement, seven tests were positive. After 15 minutes at 60°C. with complement, seven tests (in 4 cases) were hemolytic, though in low titres. Of the 3 negative tests, two were drawn 18 and 19 days after the burn.

Fourteen control sera stored from 199 to 660 days gave the following results: 14 were positive when non-inactivated serum was used. After inactivation at 56°C. with complement, four had hemolysins. There was no hemolytic activity after inactivation at 60°C.

From these studies it appears that a sheep cell hemolysin is normally present in a high percentage of individuals but that this hemolysin is dissipated with ageing at room and icebox (4°C.) temperatures, but not at -20°C. Heat (56° to 60°C.) augments the disappearance of this hemolysin.

When sera of normal individuals are heated to 60°C. for 15 minutes the sheep cell hemolysin disappears even after complement is added.

In acutely burned individuals, especially extensive burns, this hemolysin resists ageing at icebox temperatures (4°C.) for indefinite periods (up to 150 days) and likewise this hemolysin remains when such sera are heated for 60°C. for 15 minutes in the majority of cases, albeit the titre is reduced.

B. Improved Precipitin Test Procedures

In previous reports, the ring precipitin test was carried out in 4 mm. (inside diameter) tubes with 0.1 ml of antigen overlaying 0.1 ml of antibody (1.5 to 2 cm. column each). The incubation was at room temperature and the readings were made after approximately one hour. The proficiency of this test depended upon a high titred antisera for demonstrating "toxin" and a highly toxic sera to demonstrate antibodies (table I and table II). It soon became evident that when highly active sera were not available the percent of positive reactors fell off sharply.

It was reasoned that if one allowed the reactions to proceed for longer periods of time, perhaps the percentage of positive reactors would increase. It was also reasoned that if the antisera underlay had a somewhat higher specific gravity than the overlay, the diffusion of the ring formed at the contact surface would be delayed. To this end, for screening, the underlay was diluted in saline 1:2 whereas the overlay was diluted 1:4 and plasma was used as an underlay in the same dilutions as for sera. The time for reading was over a period of 24 hours and occasionally for longer periods.

Another modification was to add A and B specific blood substance (equal parts) to the antisera and incubate at room temperature for 15 minutes to minimize the possibility of isoagglutinations.

Results

Screening of Convalescent Burn Sera

In screening samples of convalescent burn sera against recent acute burn sera, positive reactions were obtained in 16 out of 27 tests (table VII). To obtain these results the convalescent burn sera was diluted 1:2 with 0.9% saline and overlaid with a 1:4 dilution (saline) of the acute burn sera. These reactions generally occurred within 6 to 12 hours and varied in titres from 1:8 to 1:64.

Replacing the acute burn sera with normal sera in three different tests gave no positive precipitin reactions with the convalescent burn sera; however, in replacing the convalescent sera with normal sera one positive reaction was obtained in 14 tests.

The acute burn sera was taken from individuals prior to death and in some cases within one half hours after death. Tests showed (2 cases) that there was no real difference between serum taken before death and that taken shortly after death (table VIII). However, if 2 to 3 hours passed

after death before the blood was drawn, no precipitin reactions were obtained (2 cases). Likewise, blood removed from individuals who had died shortly after burning gave no positive reactions (1 case).

Screening of Convalescent Burn Plasmas

It was noted that a positive precipitin ring test was obtained when acute burn sera was used to overlay convalescent burn plasma. To test this observation, an experiment was designed to compare the reactivities of serum to plasma from the same convalescent burn donor. These results are given in Table IX.

The acute burn sera used in this experiment gave positive reactions with all the convalescent burn plasma tested. The reactions were no better than those obtained with sera, and in fact, somewhat slower--requiring up to 20 hours before detection.

However, it was possible to pick up weak reactions in three plasmas tested that did not occur in the sera tested from the same donors. The affect of blood group specific substances on plasma ability to detect or increase strength of precipitin ring reactions will be discussed below.

Through an oversight no control plasmas were run with this experiment; however, in a later experiment control plasmas were overlaid with acute burned sera. In 42 such tests all but 3 were negative after three hours incubation, but in one trial of 25 acute overlaying so called 'normal' plasmas, positive reactions occurred in a high percentage (84%) after 26 hours incubation at room temperature. The source of these 'controls' were mainly from the lowest income groups, and its significance must be ascertained (table XII).

In order to exclude the possibility of interference with blood group specific substances (BGSS) a combination of A and B substances* was added to the plasma and incubated for 15 minutes at room temperature. These experiments showed that the addition of BGSS to the plasma of convalescent burns increased the strength of the reaction, and in most cases the time of the reaction was decreased to a substantial degree. The BGSS was used to replace saline in the dilution to 1:2 of the convalescent plasma. It was also noted that in some cases where BGSS was added to normal plasma, which had been used as controls over the convalescent burn plasmas, the tendency to produce false positive reactions was reduced (see table X). In subsequent experiments BGSS was used routinely and the incubation time was reduced to 3 hours.

Screening of Acute Human Burn Sera

Since the samples of convalescent burn sera which gave positive results with acute burn sera were small (2 to 5 cc each) they were quickly used up in the various tests which were done. Thus, it became necessary to rely upon convalescent burn plasma in screening recent human burns (from Cook County Hospital). The results obtained from one screening program are given in table XI.

In this experiment convalescent burn plasma 09560 diluted 1:2 with BGSS was used as the underlay. Seventeen positive results were obtained from 24 tests (71% positive). In three negative cases the burns were sustained 39 days previously.

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* Merck, Sharp & Dohme Company

The 24 acute burn sera overlaying a pool of normal plasmas (3) gave positive results in only three cases (12%), whereas, normal sera (7 samples) overlaying convalescent burn plasma 09560 were all negative. In 58 normal sera overlaying other normal plasmas 57 were negative (see table XII).

Table XII summarizes all of the precipitin ring tests performed by the "improved" techniques. It must be appreciated that there were limitations in the availability of the test materials, i.e., acute, convalescent and normal bloods. This accounts for the variability of results. Use was made of blood obtained postmortem in acutely burned cases since "acute" burn sera from living patients can only be obtained in small amounts. This source has limitations since blood must be drawn shortly after death (within one hour) and if there had been extensive transfusions the possibility of not being able to detect "toxin" becomes apparent.

Convalescent blood is more readily obtainable, but mainly as plasma and it is for this reason that plasma was assayed for precipitin tests.

In the testing procedures therefore, the materials available at the time were used. As a rule, when screening convalescent sera, one or two acute sera were used throughout.

Similarly when testing acute burn sera, one or two convalescent sera or plasma were used. There was rarely enough material to run all acute sera against all convalescent plasma or sera.

In table XII it will be noted that there were variations in the percentage of positive reactors depending upon the potency of the "acute" or "convalescent" samples. Under "ideal" conditions, which includes not only extent of burn but also the critical time after burning, a high percentage of reactors were detected. Attempts are being made to produce standardized "toxin" and "antitoxin" for more repeatable results.

The use of blood type specific substances and prolonged incubation at room temperature have improved the sensitivity of this test, but it may also increase the percentage of false positive reactions. A disturbing feature in the prolonged incubation (8 to 24 hours at room temperature) is that in several experiments the so called 'normal' plasmas, when overlaid with acute burn sera developed ring precipitates. These 'normal' plasmas were drawn from individuals of very low socio-economic groups. It is difficult at this time to ascertain whether or not the false reactions were due to the fact that these 'normal' plasmas were from individuals who had sustained injuries all their lives, and had developed autoantibodies or whether in some special instances the acute burn plasma contains proteolytic enzymes which react with protein (proteolysins) to cause precipitation. The plasma containing sodium citrate may inhibit the proteolytic enzyme inhibitor, thus allowing the proteolysis to continue. Inactivation of the plasma (56°C. for 30 minutes) did reduce the titres and/or negate them.

In one experiment the precipitin reactions took place at 4°C. using acute burn sera and immune plasma. This experiment was done to exclude properdin, which does not react at this temperature. Similarly, the antisera failed to react with C reactive protein although the acute sera reacted to C reactive protein antibody.

Current Studies

TABLE I

TIME OF APPEARANCE OF "TOXIN-ANTITOXIN" IN BURNED HUMAN SUBJECTS

(1st and 2nd Degree)

Time	% Burn	No. Cases	"Cytolysinogens-Cytolysins"			Precipitinogens-Precipitins		
			No. Positive Cases	No. Tests	No. Positive Tests	No. Positive Cases	No. Tests	No. Positive Tests
0-24 hours	3-20	6	0	9	0	0	6	0
25-48 hours	2-15	11	1	27	2	1	12	1
49 hours-6 days	3-12	7	4	23	7	2	6	2
7-35 days	3-25	8	1	16	2	0	8	0
36-240 days	3-10	5	0	17	0	0	8	0

Total cases: 24 (15 months-63 years)

Current Studies

TABLE II

TIME OF APPEARANCE OF "TOXIN-ANTITOXIN" IN BURNED HUMAN SUBJECTS

(1st, 2nd, and 3rd Degree)

Time	% Burn	No. Cases	"Cytolysinogens-Cytolysins"			Precipitinogens-Precipitins		
			No. Positive Cases	No. Tests	No. Positive Tests	No. Positive Cases	No. Tests	No. Positive Tests
0-24 hours	3-65	2	0	4	0	0	2	0
25-48 hours	2-28	2	1	4	2	0	1	0
49 hours-6 days	5-65	7	4	15	9	4	7	4
7-35 days	2-10	10	0	16	0	0	12	0
36 days-2 1/2 years	3-65	33	13	87	13	19	36	19

Cases 0 to 35 days: 15 (14 months-60 years)

TABLE III

METHODS USED IN ATTEMPTS TO DETECT ANTI-SHEEP HEMOLYSINS

(1) 0.25 cc Serum, 0.1 cc 2 percent sheep cell suspension. Incubate 1 hr. at 37°C., add complement diluted 1:10, 1:20, 1:40. Incubate 1 more hour at 37°C.	(2) 0.25 cc serum, 0.1 cc packed sheep cells. Incubate 1 hr. at 37°C. Add complement diluted 1:10, 1:20, 1:40. Incubate 1 more hour at 37°C.	(3) 0.25 cc serum, 0.1 cc 2 percent cell suspension, add complement first incubate 2 hrs. at 37°C.
(4) 0.25 cc serum, 0.1 cc packed cells, add complement 1:10, 1:20, 1:40 first. Incubate 2 hrs. at 37°C.	(5) 0.25 cc serum, 0.1 cc packed cells Incubate 1 hr. at 37°C., add complement 1:10 plus 0.5 cc saline. Incubate 1 more hr.	(6) 0.25 cc serum, 0.1 cc 2 percent cell suspension. Incubate 1 hr. at 37°C. add complement 1:10. Reincubate, after adding 0.5 cc saline, 2 hrs. at 37°C.
(7) 0.5 cc serum, 0.05 cc of packed cells. complement 1:10 added first. Incubate 2 hrs. at 37°C.	(8) 0.5 cc serum, 0.05 cc of packed cells Incubate for 2 hrs. at 37°C.	(9) 0.5 cc serum, 0.05 cc of packed cells. Incubate 2 hrs. at 37°C., add comple- ment 1:10. Reincubate for 2 hrs. at 37°C.
(10) 0.5 cc serum, 0.05 cc of packed cells. Incubate for 1 hr. at 37°C. No complement. Add 3 cc of saline and centrifuge.	(11) 1 cc of serum, 0.01 cc of packed cells. Incubate 2 hrs. at 37°C. No complement. Add 3 cc of saline and centrifuge	(12) 0.25 cc serum, 0.1 cc of 2 percent cell suspension. Incubate at 37°C. for 2 hrs. Add complement 1:10 and reincubate 2 hrs. at 37°C.
(13) 0.25 cc serum, 0.1 cc 2 percent cell suspension. Incubate at room temperature for 2 hours. Add complement 1:10. Incubate 2 more hrs.		

Current Studies

TABLE IV

RESULTS OF ANTI-SHEEP HEMOLYSINS FOUND IN CONTROL SERA

Age of Serum Days	Total Tests	Number Positive	Percent Positive - Titres 1:4	1:8	1:16	1:32	Percent Negative
Active No Complement							
Days 1-7	56	56 100%	1 20%	2 3%	17 31%	36 64%	
Days 8-14	7	5 71%		4 80%	1 20%		2 29%
Days 15-24	14	9 64%	2 22%	1 11%		6 66%	5 36%
Days 30-73	15	3 20%		1 33%		2 66%	12 80%
TOTAL	92						
Active with Complement							
Days 1-7	56	56 100%		4 7%	8 14%	44 78%	
Days 8-14	9	7 88%			2 29%	5 71%	2 22%
Days 15-24	14	13 92%	3 23%	3 23%	1 7%	6 46%	1 8%
Days 30-73	13	2 15%	1 7%		1 7%		9 85%
Inactive 1/2 hr. at 56° - No Complement							
Days 1-7	57	1 2%					56 98%
Days 8-14	11	0					11 100%
Days 15-24	14	0					14 100%
Days 30-73	16	0					16 100%
TOTAL	98						
Inactive 1/2 hr. at 56° - With Complement							
Days 1-7	57	42 73%	4 8%	6 14%	9 20%	23 54%	15 27%
Days 8-14	11	6 54%		2 33%		4 66%	5 46%
Days 15-24	14	7 50%	2 29%	3 42%	1 14%	1 14%	7 50%
Days 30-73	13						13 100%
TOTAL	95						
Inactive 15 min. at 60° with or without complement							
Days 1-7	11	0					11 100%
Days 8-14	4	0					4 100%
Days 15-24	9	0					9 100%
Days 30-73	2	0					2 100%
TOTAL	26						

Current Studies

TABLE V

RESULTS OF ANTI-SHEEP HEMOLYSINS FOUND IN BURN SERA

Age of Serum Days	Total Tests	Number Positive	Percent Positive - Titres 1:4	1:8	1:16	1:32	Percent Negative
Active - No Complement							
Days 1-7	2	2 100%				2 100%	
Days 8-14	2	2 100%				2 100%	
Days 15-24	4	4 100%				4 100%	
Days 30-73	11	10 91%				10 91%	1 9%
Days 74-150	5	5 100%		1 20%	1 20%	3 60%	
TOTAL	24						
Active - Plus Complement							
Days 1-7	2	2 100%				2 100%	
Days 8-14	2	2 100%				2 100%	
Days 15-24	4	4 100%				4 100%	
Days 30-73	11	11 100%				11 100%	
Days 74-150	5	5 100%			2 40%	3 60%	
No Complement - 1/2 Hr. at 56°C.							
Days 1-7	2	0					2 100%
Days 8-14	2	0					2 100%
Days 15-24	8	0					8 100%
Days 30-73	13	0					13 100%
Days 74-150	6	0					6 100%
TOTAL	31						
Plus Complement - 1/2 Hr. at 56°C.							
Days 1-7	2	2 100%				2 100%	
Days 8-14	2	2 100%				2 100%	
Days 15-24	8	8 100%		1 8%	3 42%	4 50%	
Days 30-73	13	13 100%	1 10%	3 23%	4 30%	5 37%	
Days 74-150	6	5 84%		1 16%	2 33%	2 33%	1 16%

Current Studies

TABLE V (Continued)

RESULTS OF ANTI-SHEEP HEMOLYSINS FOUND IN BURN SERA

Age of Serum Days	Total Tests	Number Positive	Percent Positive - Titres 1:4	1:8	1:16	1:32	Percent Negative
No Complement - 15 min. at 60°C.							
Days 1-7	2	0					2 100%
Days 8-14	2	0					2 100%
Days 15-24	3	0					3 100%
Days 30-73	6	0					6 100%
Days 74-150	5	0					5 100%
TOTAL	18						
Plus Complement - 15 min. at 60°C.							
Days 1-7	2	2 100%		2 100%			
Days 8-14	2	2 100%	1 50%	1 50%			
Days 15-24	3	2 66%	1 50%	1 50%			1 33%
Days 30-73	6	4 66%	3 65%	1 22%			2 33%
Days 74-150	5	3 60%	2 66%	1 33%			2 40%

These tests were all performed on four acutely burned individuals having from 45 to 65% (2nd and 3rd degree) burns

Current Studies

TABLE VI

COMPARISON OF ANTI-SHEEP HEMOLYSINS IN "BURN" AND NORMAL SERA
Stored at -20°C.

DAYS OF STORAGE	TOTAL TESTED	HEMOLYSINS NUMBER	HEMOLYSINS PERCENT	METHOD
Burn Sera		15	100%	Active - No Complement
360 to 455 Days	15	15	100%	Active - Plus Complement
	15	0	0%	Inactivated 1/2 Hr. at 56°C. No Complement
		7	46%	Inactivated 1/2 Hr. at 56°C. Plus Complement
(7 cases *)	15	0	0%	Inactivated 15 min. at 60°C. No Complement
		7	46%	Inactivated 15 min. at 60°C. Plus Complement
Controls		14	100%	Active - No Complement
199 to 660 Days	14	14	100%	Active - Plus Complement
	14	0	0%	Inactivated 1/2 Hr. at 56°C. No Complement
		4	28%	Inactivated 1/2 Hr. at 56°C. Plus Complement
(14 cases)	14	0	0%	Inactivated 15 min. at 60°C. No Complement
		0	0%	Inactivated 15 min. at 60°C. Plus Complement

* 8 - 23% burns (1st to 3rd degree) blood drawn 7 to 38 days after burning; ages 3 to 54 years.

Current Studies

TABLE VII

RESULTS OF PRECIPITIN TESTS IN CONVALESCENT BURN SERA

CONVALESCENT SERA	% BURN	DEGREE	TIME AFTER BURN	RESULTS OF TEST
Brown 1	47	2, 3	3 mos.	-
Brown 2	47	2, 3	3 mos.	-
Martin 1	40	3	3 mos.	-
Martin 2	40	3	3 mos.	-
Strickland 1	50	2, 3	4 mos.	+
Strickland 2	50	2, 3	4 mos.	+
Anthony 1	60	2, 3	5 mos.	+
Anthony 2	60	2, 3	5 mos.	+
60594	25	?	less than 1 yr.	+
Clark	50	2, 3	1 yr.	-
Harvey	25	?	1 yr.	-
Wright	small deep	3	1 yr.	+
11962	50	3	1 yr.	+
60601	40	?	1 yr.	+
66784	30	2, 3	1 yr.	-
Hunt	40	1,2,3	2 yrs.	+
Miller	15	?	2 yrs.	-
Tischardt	38	2, 3	2 yrs.	-
Boller	40	3	3 yrs.	-
Iffert	85	2, 3	3 yrs.	+
Sellery	85	2, 3	3 yrs.	+
Whitehurst	35	2, 3	3 yrs.	-
15838	35	2, 3	3 yrs.	+
66805	33	2, 3	3 yrs.	+
12174	35	?	4 yrs.	+
09560	30	?	5 yrs.	+
Doubets	35	2, 3	7 yrs.	+

Current Studies

TABLE VIII

PRECIPITIN RING TESTS WITH ACUTE BURN SERA
PRE AND POST MORTEM

(Serum over serum and dilutions made in saline.)
Experiment to test reactivity of Simpson's serum one week after burning (10-31-60), three hours before death (11-5-60) and 30 minutes after death (PM).

All Simpson sera diluted 1;4 and used to overlay donor sera diluted 1:2.
1 = Simpson (10-31), 2 = Simpson (11-5), and 3 = Simpson (PM)

DONORS	TIME IN HOURS								
	2			4			6		
	1	2	3	1	2	3	1	2	3
1. #47755	-	-	-	-	-	-	-	-	-
2. #11962	±	±	±	+w	+w	+w	+	+	+
3. #66784	-	-	-	±	-	-	+w	-	±
4. Iffert	±	+w	+w	+w	+w	+w	+	+	+
5. #66805	+w	+w	+w	+	+	+	+s	+s	+s
6. Tischardt	±	-	-	-	-	-	-	-	-
7. Control 2059	-	-	-	-	-	-	-	-	-
8. TC Serum (control)	-	-	-	-	-	-	-	-	-
9. Sellery	±	-	±	±	-	±	+w	-	±

(Serum over serum and serum over plasma; dilutions are made in saline.)
Experiment to test Green serum (PM*) and before death on several sera and plasma.

All overlaying sera diluted 1:4 and all underlaying sera or plasma diluted 1:2 with saline.

	TIME IN HOURS					
	0.5	3.5	5	8	10	12
Green (PM) 1:4 Serum 60601 1:2	±	+	+	+w	±	-
Same Serum 60594	+	+	+	+w	+w	+w
Same Normal Serum 2064	All Negative					
Same Wilma Serum (Normal)	All Negative					

* Blood drawn immediately after death.

Current Studies

TABLE VIII (Continued)

PRECIPITIN RING TESTS WITH ACUTE BURN SERA
PRE AND POST MORTEM

Experiment to test Green serum (continued).

	TIME IN HOURS					
	0.5	3.5	5	8	10	12
Green (BD) Serum 60601	-	-	+w	+	+w	+w
Same Serum 60594	+w	+	+	+	+d	+d
Same Normal Serum 2064	All Negative					
Same Wilma Serum	All Negative					
Normal Serum 2064 Serum 60601						
Normal Serum 2064 Serum 60594	ALL NEGATIVE AT ALL TIMES					
Normal Serum 2064 Normal Serum 2064						
Normal Serum 2064 Wilma Serum (Normal)						
Green (PM) Plasma 60601	+w	±	±	±	±	-
Green (PM) Plasma 60594	±	±	±	±	±	+w
Green (PM) Normal Plasma 8	All Negative					
Green (PM) Normal Plasma 5	All Negative					
Green (BD) Plasma 60601	±	±	±	+w	+w	+
Same Plasma 60594	±	±	±	+w	+w	+
Same Normal Plasma 8	All Negative					
Same Normal Plasma 5	All Negative					
Normal Serum 2064 Plasma 60601						
Same Plasma 60594	ALL NEGATIVE AT ALL TIMES					
Same Normal Plasma 8						
Same Normal Plasma 5						

Current Studies

TABLE VIII (Continued)

PRECIPITIN RING TESTS WITH ACUTE BURN SERA PRE AND POST MORTEM

Experiment to test Simpson (PM) over normal plasma

	TIME IN HOURS					
	0.5	3.5	5	8	10	12
Simpson Normal Plasma 8						
Same Normal Plasma 5	ALL NEGATIVE AT ALL TIMES					
Same Plasma 60601	±	-	-	-	+	+

PM = Post Mortem

BD = Before death

+w = Weak band

+d = Diffuse band

+s = Strong band

Current Studies

TABLE IX

PRECIPITIN RING TESTS OF ACUTE AND CONVALESCENT BURN SERUM AND PLASMA

(Serum over serum and serum over plasma; dilutions made in saline.)

Simpson (PH) used as toxin, diluted 1:4 and used as overlay of the following sera and plasmas (all diluted 1:2).

TEST	TIME IN HOURS						
	1	2.5	4	11	20	24	26
#11962 Serum No Plasma	±	±	+w	+w	+*	+w,*	+w,d
#66784 Serum	-	-	-	-	-	-	-
#66784 Plasma	-	-	-	+w	+	+w	nr
Iffert Serum	-	+w	+w	+w,*	+w,d,*	+w,d,*	+w,d,*
Iffert Plasma							
#66805 Serum	+	+s	+w	+*	+w,d	+w,d,*	nr
#66805 Plasma	-	-	-	-	±	±	+w
Tischardt Serum	-	-	-	-	-	-	-
Tischardt Plasma	-	-	-	-	+w	+w	+w
#1-12174 Serum	+	+s	+w	+*	+*	+w,d,*	nr
#1-12174 Plasma							
#60601 Serum	+w	+w	+	+*	+*	+w, d,*	nr
#60601 Plasma	-	-	+w	+w	+s	+s	+
#95581 Serum	-	-	-	-	-	-	-
#95581 Plasma	-	-	+w	+	+s	+s	+
#22125 Serum	+w	+w	+w	+w	+w,*	+w,d,*	nr
#22125 Plasma	-	-	+w	+w	+	+*	+w
#60594 Serum	+s	+s	+*	+*	+*	+d,*	nr
#60594 Plasma	-	-	+w	+w	+w	+w	+*
Miller Serum	-	-	-	-	-	-	-
Miller Plasma	-	-	-	-	±	±	±
#15838 Serum							
#15838 Plasma	-	-	+w	+	+*	+*	+*
#09560 Serum							
#09560 Plasma	-	-	-	+	+	+w	+w
#95593 Serum							
#95593 Plasma	-	-	-	+w	+w	+w	±

Current Studies

TABLE IX (Continued)

PRECIPITIN RING TESTS OF ACUTE AND
CCONVALESCENT BURN SERUM AND PLASMA

CONTROL

Tissue Culture Type Serum — NEGATIVE AT ALL TIMES
Pooled Normal Human Serum

NO CONTROL ON PLASMAS

COMMENTS:

Sera: 13 donors and 2 controls. 1 control negative and one positive
Of the 13 donors 7 were negative for 25 hours.
At one hour, 8 were negative or ± and 5 were positive
At two hours, 7 were negative or ± and 6 were positive
At five hours 7 were negative or ± and 6 were positive
At 11 hours 6 were negative or ± and 7 were positive
At 20 hours there was no change
Optimum at 6 to 11 hours

Plasmas: 12 donors and no controls
At 2 hours 12 were negative
At 5 hours 4 were positive and 8 were negative
At 11 hours 7 were positive and 6 were negative or ±
At 20 to 24 hours 10 were positive and 2 were ±
Optimum 20 to 24 hours

Serum over Plasma seems to be better reactant than Serum over Serum.

+s = Positive strong
+d = Positive diffuse
+w = Positive weak
* = Double bands
nr = Not read

Current Studies

TABLE X

PURPOSE: To test Simpson and Burton against an immune plasma diluted 1:2 with BGSS at various concentrations.

MATERIALS: Simpson PM, Burton aged 1 day, Normal serum is pool of Mt. Sinai Hospital sera, Normal plasma 1921, immune plasma 09560.

ANTIGEN	2 HOURS	4 HOURS
Simpson 09560 1:2 saline		
1:4	+	+s
1:8	+w	+
1:16	-	+w
1:32	-	-
Simpson 09560 1:2 BGSS*		
1:4	+s	+s
1:8	+	+s
1:16	-	+
1:32	-	+w
Simpson 09560 1:2 BGSS 1:5		
1:4	+	+s
1:8	-	+
1:16	-	-
1:32	-	-
Simpson 09560 1:2 BGSS 1:10		
1:4	+	+
1:8	-	+w
1:16	-	-
1:32	-	-
Simpson 09560 1:2 BGSS 1:25		
1:4	+	+w
1:8	-	+
1:16	-	-
1:32	-	-
Simpson 09560 1:2 BGSS 1:50		
1:4	-	+
1:8	-	+w
1:16	-	-
1:32	-	-

A series identical to the above was made with normal plasma replacing the immune plasma--all were negative. Also normal sera was used to replace the antigens--in all cases this also was negative.

* Blood Group Specific Substances A & B

Current Studies

TABLE XI

RESULTS OF PRECIPITIN TESTS IN ACUTE BURN SERA

ACUTE BURN SERA	% BURN	DEGREE	TIME AFTER BURN	RESULTS OF TEST
Norvell BD	65	2, 3	3 hrs.	+
Burton BD	45	3	1 day	+
Norvell PM	65	2, 3	1 day PM	-
HB 17 (Ignasek BD)	65	2, 3	2 days	+
HB 18	10	2	2 days	+
HB 20 (Burton BD)	45	3	2 days	+
HB 21	15	2, 3	2 days	+
HB 22	20	3	2 days	+
HB 23	20	2, 3	2 days	+
HB 24	20	2, 3	2 days	+
Green (PM)	48	2, 3	3 days PM	+
Burton PM	45	3	4 days	-
HB 3	25	2	4 days	+
Turner	15	3	5 days	+
HB 1b	10	2	5 days	-
HB 5	10	3	5 days	+
HB 7	10	3	5 days	-
Ignasek (PM)	65	2, 3	2 weeks PM	+
Simpson	65	2, 3	2 weeks PM	+
HB 19	47	2, 3	39 days	-
HB 19b	47	2, 3	50 days **	+
HB 25	60	2, 3	5 mos.	-
HB 26	35	2, 3	6 mos.	-
HB Hinton	35	2, 3	6 mos. *	+

* Skin grafted

** Died 1 month later

Current Studies

SUMMARY TABLE XII

PRECIPITIN RING TESTS (IMPROVED METHOD)
WITH ACUTE AND CONVALESCENT BURN SERA AND PLASMA

METHOD	TESTS	NO. POS.	NO. NEG.	% POS.	% NEG.	TIME
SCREENING OF CONVALESCENT SERA						
Serum/Serum						
Acute/Convalescent	27	16	11	58	42	3 & 22 Hrs.
Normal/Convalescent	3	0	3	0	100	3 & 22 Hrs.
Acute/Normal	14	1	13	7	93	3 & 22 Hrs.
Serum/Plasma						
Acute/Convalescent	12	12	0	100	0	3 & 23 Hrs.
Normal/Convalescent	7	0	7	0	100	3 & 36 Hrs.
Acute/Normal	15	3	12	20	80	3 & 22 Hrs.
SCREENING OF ACUTE BURNS						
Serum/Serum						
Acute/Convalescent	7	7	0	100	0	6 Hrs.
Normal/Convalescent	12	0	12	0	100	22 Hrs. +
Acute/Normal	3	0	3	0	100	22 Hrs. +
Serum/Plasmas						
Acute/Convalescent	24	17	7	71	29	3 Hrs.
Normal/Convalescent	7	0	7	0	100	3 Hrs.
	16*	0	16	0	100	6 Hrs.
	15*	1	14	7	93	6 Hrs.
Acute/Normal	25	3	22	12	86	3 Hrs.
	25	21	4	84	16	26 Hrs.
	17	17	17	0	100	3 & 32 Hrs.
Normal/Normal	58	1	57	2	98	3 Hrs.
	30	1	29	3	97	22 Hrs.

* Stored at -20°C.

II. "TOXIN-ANTITOXIN" PHENOMENA IN BURNED OR INJURED GERMFREE RATS AND MICE

It is the majority opinion that the cause of death, especially late death, following severe burns is due to secondary infection and septicemia. At the First International Congress on Research in Burns, held in Washington, D. C., on September 19-22, 1960, emphasis was centered about the large variety of organisms isolated both locally and from the blood of burned individuals, the treatment by antibiotics, drug resistance, etc. Pseudomona was reported as being isolated frequently both from the open lesions and from the blood stream especially in cases of fatal outcome. There were no cases reported in which the organisms isolated from the blood stream had developed resistance to all the available antibiotics. In one report from Mexico where one series of burned subjects were treated with antibiotics while another series was not, the difference in the mortality rate was not statistically significant. This report bears further scrutiny as to the antibiotics used and method of administration.

Some of the significant data presented at this meeting relevant to this discussion was that comparing the mortality from burns before the advent of antibiotics, the modern concept of electrolytes, colloids, nutrition, etc. (before 1947) and after the introduction of antibiotics and modern methods of burn therapy, there has only been a 5% drop in mortality albeit the patients live longer, i.e., from the former average of 4.5 days to the present average of 14.5 days. At present as before, it is still true that when 50% of the body surface is burned the mortality is approximately 50%.

Unquestionably septicemia must play an important role in late deaths following burns--but it can only be one of many factors, since intensive antibiotic therapy has not significantly reduced the mortality. One such other factor is the toxemia resulting from degradation products of the injured tissue. As long as the local wounds remain open, degradation products continue to be absorbed into the circulation and give rise to a toxemia not necessarily of bacterial origin. The effect of this toxemia on the RES and other systems may be profound and ultimately contribute to the death of the patient.

Studies from this laboratory have been reported that in fact substances have been isolated from burned skin diffusates, by a sterile *in vivo* method circumventing the circulation that are toxic and lethal to mice and rats in acute or chronic experiments. This evidence favors a non-bacterial toxin derived from burned skin. To further explore the possible role of infection in death following thermal injury, the burning of germfree animals was resorted to and compared with normal animals. Arrangements were made by the Office of Naval Research in Washington and Chicago for a joint study to be done at the Lobund Institute for germfree animals at Notre Dame University and the University of Illinois in Chicago.

This study was designed to answer the following questions: (1) are germfree animals as susceptible to thermal injury as are normal animals, and, (2) is there serologic evidence for the existence of antigen-antibody complexes in the blood of germfree animals.

METHODS

Germfree and normal rats (Wister) of comparable age and weight were burned by the hot plate method (temp. 250° to 350°C. 20 sec. to 10 minutes) for about 20% of their body surface (skin of back). Blood was drawn before burning, one hour after burning and six months after burning. The following serological tests were performed: For Cytolysinogens-cytolysins: A 2% suspension of washed RBC from acutely burned rats was added to an equal volume of serialy diluted sera (0.1 ml) from burned rats (acute and chronic) plus one unit of guinea pig complement (0.5 ml of a 1:10 dilution) and incubated for 24 hours. For Precipitinogens-precipitins: The sera of acutely burned rats were overlaid (ring test) with serial dilutions of the sera of rats burned six months previously (when titrating for antibody) or vice versa (when titrating for antigen). 0.1 ml of each test material was used.

In all instances corresponding nonburned rat RBC and sera were used as controls. Germfree (GF) and normal (NGF) rat RBC and sera were interchanged both for burned and nonburned rats. All serological procedures were performed in code by a serologist (W.S.) who was not involved in the burning of the animals.

Germfree and normal mice of different weights were scalded by the immersion or basket techniques in hot water (70°C.) and the amount of heat expressed as LD_{50} in seconds was determined. In a limited number of animals, blood was drawn before and after (24 hours) burning and 4 to 6 months after burning. Serologic studies as described for rats were repeated for the mice.

RESULTS

Rats

Table I gives the results of the serological testing of the blood of germfree rats drawn 24 hours after burning (250°-350°C., 10-20 sec.). Blood from the same rats was drawn 24 hours before burning and served as controls. It will be noted that no hemolysinogens or precipitinogens could be detected when the sera of burned and healed germfree rats were used as antibody, but positive results were obtained with the sera of burned and healed normal rats. It is of interest that when RBC of acutely burned rats was added to sera of acutely burned rats (autologous or homologous) plus complement, hemolysis did take place. Nonburned germfree or normal RBC or sera gave negative results.

In table 2 the titrating for antibody was made by serialy diluting the sera of the chronically burned rats and maintaining the acute sera undiluted, the reverse of the above. It will be noted that cytolysins were detected in some of the animals and then in low titre. It must be added however that in two of the animals ulcers of the skin were still in evidence (one negative reactor and one 1:1 titre). The germfree animals healed at a slower rate than the nongermfree animals. Control sera and RBC of nonburned germfree or normal animals were negative.

Mice

Mortality Studies - Table 3 compares the LD_{50} for germfree and normal mice. Each point (LD_{50}) represents at least 20 animals with four groups of five burned at different times. The data in the table are significant between the germfree and normal animals. It is evident that the germfree mice are more sensitive to heat than the normal mice.

Serology - In a limited number of germfree and normal mice studied serologically, both antigen and antibody complexes were detected. For the precipitins at least, the normal mice developed higher titres than did the germfree mice.

The RBC and sera of nonburned mice occasionally reacted with the sera of chronically burned mice but when this occurred the titre was usually lower than for the experimental animals.

DISCUSSION AND SUMMARY

Germfree animals are more sensitive to thermal stress than normal animals. Since the tissues of normal mice and rats are considered sterile, and in germfree animals, the hollow organs as well are germfree, the shock and death that results following burning cannot be of bacterial origin under the conditions of this experiment. The highest mortality following scalding of mice as presented (germfree and normal) occurred in the first 48 hours--but deaths did occur up to twelve days or as long as these acute experiments were followed. Deaths will occur for as long as 30 days following burning.

Serological evidence of antigen complexes appear in the blood of acutely burned normal and germfree rats and mice. Antibody complexes were detected with healing of the local injury. In germfree rats either because they have less antibody building vitality or because the local wounds are slower in healing, the antibody titres were lower or absent as compared to normal animals. The antigen-antibody complexes in question in the two types of animals are probably similar because they cross-react. These are but preliminary studies and will need further elaboration.

Germfree Studies

TABLE 1

SEROLOGY IN GERMFREE BURNED RATS
AFTER 24 HOURS

	CYTOLYSINOGENS		PRECIPITINOGENS	
Rat No.	GF	NGF	GF	NGF
D-6	-*	1:4	-	1:2
D-7	-*	1:8	-	1:4
D-9	-*	1:2	-	1:4
D-17	-*	1:4	-	1:2

* + with own sera

20% BURN (250-350°C.)

TABLE 2

SEROLOGY IN GERMFREE BURNED RATS
AFTER 6 MONTHS

	CYTOLYSINS		PRECIPITINS	
Rat No.	GF	NGF	GF	NGF
B-1	1:2	-	-	-
B-3	1:1	-	-	-
B-5	-	-	-	-
B-10	-	-	-	-
B-15	1:4	-	-	-

20% BURN (250-350°C.)

TABLE 3

SEROLOGY IN SCALDED MICE AFTER 24 HOURS

	CYTOLYSINOGENS		PRECIPITOGENS	
No.	GF	NGF	GF	NGF
GF-M3	1:4	±	1:2	1:4
NGF-M6 or NGF-M7	1:2	-	1:2	1:4
NGF-M8 or NGF-M9	1:2	-	1:4	1:2

70°C. (7-8 sec.) 30% BURN

SEROLOGY IN SCALDED MICE AFTER 60 DAYS

	CYTOLYSINS		PRECIPITINS	
No.	GF	NGF	GF	NGF
GF-M1	1:4	1:2	1:2	N.D.
GF-M2	1:1	-	1:2	1:4
NGF-M10	-	-	1:1	1:4
NGF-M11 or NGF-M12	±	-	1:4	1:2

70°C. (7-8 sec.) 30% BURN

III. BASKET TECHNIQUE FOR PRODUCING STANDARD THERMAL INJURY IN MICE

The survival rate of scalded mice is an accpetable method for determining the effect of various chemical or biological agents on thermal injury. The standard technique is to anesthetize the animal and dip the lower two-thirds (up to the arm pits) in water at 70°C. for varying lengths of time. (1, 2, 3) This method, while serving well for acute experiments (up to three days), has certain limitations, particularly in chronic experiments. For example, the gangrene of the paws, tail, and scrotum, which is the rule following the above procedure, is a source of secondary infection. The possibility also exists that the urethral and anal orifices may be so injured as to be another source of infection and/or that the latter injuries may impede elimination. These complications obscure the issue being studied, namely thermal injury, and limit the effective time of the study.

The basket technique here described burns only the back of the animal (approximately 30 percent of the body surface) and has the following advantages: (1) there are no unrelated acute complications such as gangrene of paws, scrotum, or tail; (2) healing occurs under a closed scab or crust so that secondary infection is minimized; (3) chronic experiments may be carried on without unrelated chronic complications; (4) results are repeatable.

METHOD

A basket* made of stainless steel mesh (64 mesh) with a lid that can be secured firmly, was so constructed that the mouse fitted into it snugly and was secured firmly by the lid which covered chest and abdomen only.

---

* Made by Edwin Herskind, 3847 North Janssen, Chicago 13, Illinois.

The basket is so shaped that the bottom accepts the curvature of the back of the animal, and the ends are tilted upwards so that urethral and anal orifices and tail on one end and the head on the other are at a level above the anterior axillary line of the mouse. Lateral stainless steel arms project from the basket in such a manner and position that when the preparation is set into a water bath it remains fixed and the entire back of the animal up to the posterior axillary line is submerged in the water. From the two central arms, looped vertical handles project for grasping the basket.

The water bath is composed of two sections. The inner section is a stainless steel beaker 3 inches in diameter and 4-1/2 inches high which is slightly lipped. Three slots, 11/16 of an inch wide and 5/16 of an inch deep, cut out of the upper edge, allow for the overflow of water. The beaker is suspended through an opening in a thin metal plate to which it is permanently fixed. Lateral slots in the metal plate admit the central arms of the basket. The dimensions of the metal plate are such that it covers the upper edges of a standard water bath completely, thus avoiding vapors and heat issuing from the bath. A small opening caudal to the beaker admits the glass rod of a bulb-glass rod combination used to refill the beaker with hot water from the larger bath between each animal dipping. The water in the larger bath is circulated by a stirrer. The temperature of the inner section bath must be constant (±1/2°C.) and regularly checked by the stationary thermometer.

The procedure used in this study was as follows. The mice were Swiss white males, all purchased from one distributor and weighing from 18 to 22 grams (before clipping hair) and chosen so that a randomization of weights was

maintained within this range. The basket was made to fit a mouse this size snugly after the hair of the back was shaved. Because of basket variations the same basket was used throughout. The temperature of the water bath was carefully adjusted to 70°C. (±1/2°C.). The metal plate holding the beaker was adjusted to rest horizontally with the help of a carpenter's spirit level. The hair of the entire back of the mouse was clipped closely and carefully with a hair clipper (Oster head No. 40) just before scalding. The animal was placed in a glass jar containing a pledget of ether-soaked cotton which was covered by a wire mesh so that the ether-soaked cotton never touched the animal. When the respirations became slow and regular, the animal was removed and quickly placed in the basket and the lid closed, being sure that paws and ears were well above the lid and that tail, anus, urethra, and head were above the anterior axillary line. The basket was set into the inner water bath and the exposure timed with a stop watch. It is estimated that about 30 percent of the body surface is thus burned. After scalding, the basket with the animal was removed and the animal quickly taken out of the basket and rolled on dry, absorbent paper. Water was added between each dipping to maintain the proper fluid level.

As a rule there were not more than five groups of ten mice each burned at one sitting. A control group of ten mice anesthetized and mock-burned was always included. Either ten mice were burned consecutively at a given time interval or the time interval was randomized, burning five animals at a time. The time intervals of scalding were varied from 6 to 10 seconds; only one experiment was done at 11 seconds.

The first 45 experiments representing 429 mice were performed from mid-May through the first half of August (spring-summer) when the outside temperatures varied from 47° to 93°F. The mice were scalded a day or two after they arrived from the distributor. When first received, they were kept in an individual unit type air-conditioned room (68° to 74°F.). They were then brought down to the laboratory which was not air-conditioned and remained there for one to two hours for the duration of the experiment after which time they were returned to the air-conditioned room.

The second group of 58 experiments representing 556 mice was performed during November and December (fall) when the outside temperatures varied from 28° to 49°F. These animals were housed and experimented upon in newly constructed animal quarters with centrally controlled air conditioning (temperatures 72° to 74°F.). The animals were acclimated to these rooms for one week before scalding.

After scalding, as described above, the animals were observed daily for up to 90 days. The nature of the local lesions and the mortality rates were recorded.

RESULTS AND DISCUSSION

Description of Lesions

For the first day following the scalding there were either no gross lesions of the skin or scattered small pinhead-sized red denuded areas. On palpation, a slight thickening of the injured area was noted. Gradually the skin became indurated and furrowed and was covered by the bristles of the base of the hair shafts not removed by the clippers. By the 7th to 10th day a continuous scab or crust formed with a definite line of demarcation.

At this time there were occasionally slight breaks in this crust, light to dark red in color. No evidence of discharge was observed. By the 25th to 30th day, the scabs or crusted areas fell off in sheets and as a rule left a thinly epithelialized skin with occasional small denuded lighter red areas. From the 45th to 60th day there was restitution of the skin including hair, but the pattern of the hair was irregular. Purulent discharge or suppuration was not noted. The healing proceeded under a scab by first intention so that smears or cultures were not taken. Complications not directly related to the scalding did not occur.

Mortality Rates

Table I shows the number of experiments done and the number of animals used at each burn-time for the spring-summer (May 14 to August 12) series, fall (October 28 to December 11) series and combined series. Means and standard deviations were taken for all experiments at each burn-time, for each series, and for each observational day; those for one and two days after burning appear in table II. The means for the spring-summer series do not differ from their fall counterparts at the 5% level of significance (28 comparisons). This finding indicates that the first series was confirmed by data taken in more controlled conditions, that meaningful data can be taken in the simpler installation, and that the two series may be statistically combined. From table II it is noted that in the combined data two days after burning, the mean mortality for all experiments run at 8 seconds burn-time was 0.487 or 48.7% lethality. And in the same data the corresponding figure for 9 seconds burn-time was 0.594 or 59.4% lethality, illustrating that the LD_{50} must lie between 8 and 9 seconds. The higher mortality in burned animals as compared with controls was tested for significance by exact probability (4). P values were less than 0.001 throughout, indicating thermal injury is the major cause of death.

Data for spring-summer, fall, and the combined series were plotted in percent mortality versus burn-time, one plot for each of these at 1, 2, 10 20, 30, 60, and 90 days after burning. Since efforts had been made to confine burn times close to LD_{50}, these plots are essentially linear. Least-squares linear fit was applied to each of the 21 plots; a typical one is shown in graph 1.

Analysis of variance was applied to each of these lines, comparing regression variance and deviation from regression variance. All values are significant. Spring-summer 90 day, and fall 60 and 90 day data give P values between 5% and 2%; spring-summer 20 day data show P between 1% and 0.1% (nearer latter); in all other cases $P<0.001$. The hypothesis that a curvilinear function might significantly improve the fit is rejected.

Table III gives mortality figures obtained from these least-squares lines. Because all data for a given observational day are used for each such entry, they are more reliable than single observations and progress without inversion, always increasing with burn-time and with days after burn. In the combined data at 8 seconds of burn-time it will be seen that the LD value one day after burning is 0.40 or 40% lethality and increases to 0.44 or 44% on the second day after burning but increases only to 0.55 or 55% after 10 days, illustrating the fact that the mortality rates increase very rapidly in the first 48 hours, much less rapidly following 48 hours, but do continue to increase throughout the entire observation period. A plot of the fall data is presented in graph 2.

The gross shift in slope of these mortality plots at two days suggests that one phenomenon is operating in the first 48 hours after burning, while a separate factor of thermal injury appears to account for mortality beyond that time.

Eliminating cases that died in the first 48 hours, corrected Chi Square was used to compare two-day survivors at 30 days after burning with controls. Spring-summer 7 and 8 second data failed to show 5% significance. All other cases, spring-summer, fall or combined and at all burn-times gave $P<0.001$. This high significance strongly supports the contention that mortality due to burning continues to occur for at least 30 days, making treatment feasible. Sixty and 90-day data continue the trend but without statistical significance.

A more specific purpose of this experiment was to determine LD_{50} values as guides to burn-time in future work; these are presented in table IV together with the standard error of the mean of all experiments run at the burn-time nearest the LD_{50} in question. This measure of dispersion is appropriate for contemplated work involving one burn-time only. Fluctuations in standard errors are primarily due to differences in sample size. All figures are in seconds.

The LD_{50} values in seconds of scalding ($70^{\circ}C$) under the conditions of these experiments varied with the days after burning. Thus at 1 and 2 days it is 8.55 (±0.21) and 8.35 (±0.36) seconds respectively (combined series) whereas at 30 days it is 6.35 (±0.36) seconds. It is deduced that the effects of the thermal injury per se are still at play for a period of 30 days, i.e., as long as the local wound has not healed. The fact that healing took place under a scab thus minimizing secondary infection, and the fact that only the skin of the back was burned thus excluding unrelated complications, further supports this deduction.

Preliminary data has been seen on a similar experiment now in progress using a higher scalding temperature (80°C.). The comparisons currently possible promise even smaller variance than that reported above in the fall series.

SUMMARY

1. A basket method is described for producing a standard thermal injury (scalding) in mice.

2. This method gave repeatable results in some 103 experiments embracing 985 mice.

3. The advantages of the method are that only the skin of the back is burned, healing is by primary intention, and that this preparation can be used for acute and chronic experiments.

4. Mortality rates over a 90-day period are given for scalding (70°C.) times of 6 to 11 seconds. These results are statistically analyzed.

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TABLE I

Number of Mice and Experiments According to

Duration of Scalding and Season of Year

Seconds of Scalding	Spring-Summer		Fall		Combined	
	No. of Animals	No. of Experiments	No. of Animals	No. of Experiments	No. of Animals	No. of Experiments
0	125	14	90	11	215	25
6	-	-	60	6	60	6
7	50	5	108	11	158	16
8	90	9	158	16	248	25
9	99	10	80	8	179	18
10	55	6	60	6	115	12
11	10	1	-	-	10	1
Totals	429	45	556	58	985	103

Basket Technique

TABLE II

The Observed Mean Death Rates at 24 and 48 Hours of Scalded Mice

For Varying Times of Burning

	6 Seconds		7 Seconds		8 Seconds		9 Seconds		10 Seconds		11 Seconds	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
S 1 Day	---	---	0.140	0.102	0.529	0.212	0.639	0.223	0.733	0.130	1.000	0.000
S. 2 Day	---	---	0.180	0.172	0.543	0.232	0.639	0.223	0.742	0.138	1.000	0.000
F 1 Day	0.117	0.090	0.181	0.187	0.398	0.281	0.500	0.200	0.733	0.189	---	---
F 2 Day	0.117	0.090	0.227	0.198	0.460	0.312	0.538	0.173	0.817	0.177	---	---
S & F 1 Day	0.117	0.090	0.168	0.166	0.403	0.271	0.577	0.224	0.733	0.162	1.000	0.000
S & F 2 Day	0.117	0.090	0.210	0.190	0.487	0.291	0.594	0.209	0.779	0.163	1.000	0.000

S Spring-summer series
F Fall series
S & F Combined series
M Mean mortality expressed as a decimal
SD Standard deviation
-- Not done

For number of animals see table I

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TABLE III

Rates of Mortality According to Duration of Scalding

And Time After Scalding

Derived from Least-square Lines

Seconds of Scalding	Days After Scalding						
	1	2	10	20	30	60	90
	Spring-Summer						
7	0.25*	0.27	0.32	0.45	0.45	0.50	0.64
8	0.43	0.45	0.49	0.59	0.60	0.65	0.76
9	0.62	0.62	0.66	0.73	0.76	0.80	0.87
	Fall						
7	0.23	0.26	0.43	0.56	0.61	0.68	0.68
8	0.38	0.43	0.57	0.69	0.72	0.76	0.76
9	0.54	0.60	0.71	0.82	0.83	0.85	0.85
	Combined						
7	0.23	0.27	0.42	0.55	0.57	0.63	0.66
8	0.40	0.44	0.55	0.66	0.69	0.73	0.76
9	0.58	0.61	0.68	0.77	0.80	0.83	0.85

* Rate of mortality expressed as a decimal

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TABLE IV

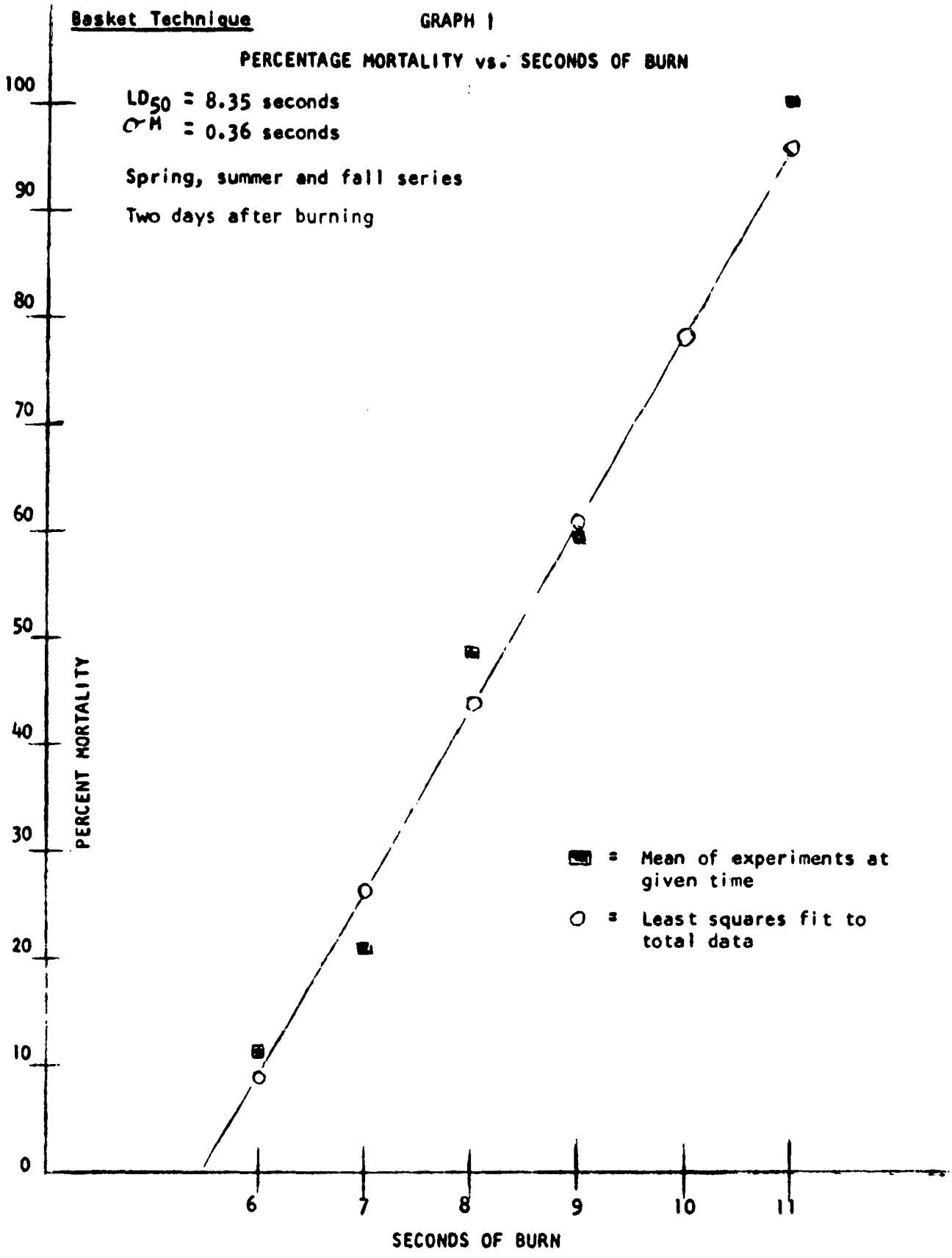
LD_{50} Values in Seconds of Scalding

(70°C.)

Derived from least-square lines.

Days After Scalding	Spring-Summer		Fall		Combined	
	LD_{50}	σ_m	LD_{50}	σ_m	LD_{50}	σ_m
1	8.37	0.62	8.76	0.46	8.55	0.21
2	8.29	0.54	8.40	0.51	8.35	0.36
10	8.06	0.04	7.50	0.04	7.62	0.42
20	7.38	0.10	6.05	0.25	6.60	0.66
30	7.32	0.62	6.02	0.42	6.35	0.36
60	6.98	0.74	4.95	0.88	5.63	0.77
90	5.77	1.22	4.90	0.88	5.26	0.80

* σ_m is the standard error of the mean of the series nearest to the given LD_{50} in seconds.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GRAPH I
PERCENTAGE MORTALITY vs. SECONDS OF BURN
LD_{50} = 8.35 seconds
σ_M = 0.36 seconds
Spring, summer and fall series
Two days after burning
100
90
80
70
60
50
40
30
20
10
0
PERCENT MORTALITY
6
7
8
9
10
11
SECONDS OF BURN
= Mean of experiments at given time
= Least squares fit to total data

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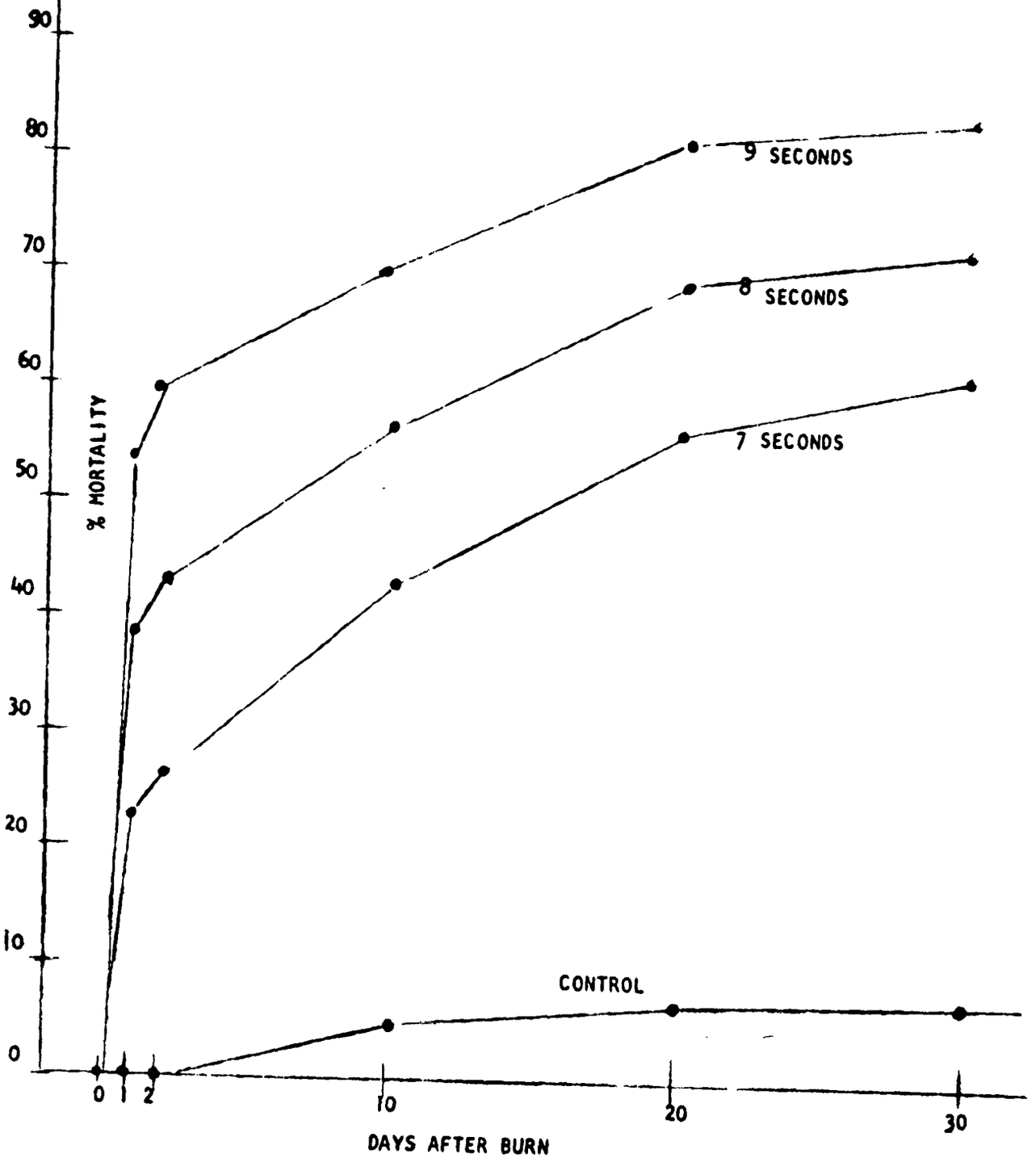
GRAPH 2

PERCENTAGE MORTALITY vs. DAYS AFTER BURN,
SHOWING BURN TIMES OF 7, 8 and 9 SECONDS AND CONTROLS

FALL SERIES



IV. PLANS FOR THE FUTURE

Based on experimental and clinical evidence that following tissue injury (thermal, chemical, radiation and trauma) degradation products resulting from tissue breakdown develop which may be toxic and harmful to the host and that with recovery there is a concommitant production of antibodies to these denatured tissue products which have the capacity of neutralizing some of the said toxic effects, therefore, it is proposed to establish blood banks for the collection, storage and processing of blood from convalescent patients who have sustained various serious injuries. These bloods are to be transfused into patients who have sustained extensive and critical injury from various origins.

Since healed injury donor blood is only limited in amount and may be irregularly localized it is recommended that each state have one central bank and that on a national basis there be one or more (east, central and west) depositories. The blood may be collected locally and a sample sent to the state center for titrating potency. The blood thus collected may be stored locally for three weeks and used locally if needed. After this time the blood is to be sent to the state center for separating plasma and storage. A certain percentage of the plasma units are to be sent to the central or regional national center for storage in the fluid or dried state or as the gamma globulin fraction. Each contributor is to be credited with the number of units of plasma it donates.

1. SOURCE OF BLOOD

Blood is to be obtained from individuals who have had extensive tissue injury (thermal, chemical, radiation, traumatic and surgical) from which they are considered recovered and with complete tissue restoration within a three year period. The following are the categories

from which blood is to be obtained:

A. Thermal or chemical burns

Full thickness skin burns of 10% or more of the body surface

B. Radiation injury

1) accidental

2) following therapy (minimum dosage will depend upon type of ionizing radiation used)

C. Post traumatic

1) Fractures

2) Severe contusions and abrasions with residual scars of 10% or more of body surface

3) Any injury associated with unconsciousness for two hours or more

D. Post surgery

Where duration of surgery was one hour or more

II. DETERMINATION OF POTENCY OF BLOOD

A. Antibody titre of plasma or serum by precipitin, hemolysin, complement fixation colloidal particle agglutination tests

B. Neutralization titres of 'toxin' by tissue culture methods (HeLa cells, etc.)

III. CLINICAL APPLICATION

The transfusion of healed injury donor blood or plasma is to be considered in individuals who have sustained 10% or more of body surface third degree and/or 25% or more of second degree burns, (thermal, chemical or radiation); in extensive trauma, fractures or following major surgery.

A. Acutely burned or injured patients

1) Transfuse with whole blood or plasma as indicated using healed injury donors instead of normal donors

2) Minimal amount of transfusion to be 125 ml. of plasma or 250 ml. of whole blood daily

3) Administer first daily, then every other day, weekly, etc., depending upon toxicity

4) Transfuse two days before skin grafting and daily thereafter until "take" is assured

5) Continue transfusion until less than 10% of the body surface is exposed

B. Radiation

Use blood or plasma from patients who have been irradiated and recovered if available, otherwise use healed injury donor blood. Follow same regime as under "A."

1) accidental

2) Following radiation treatment

a. For radiation sickness

b. As an adjunct to allow for larger radiation dosage

C. Post-trauma

1) Fractures

2) Contusions and abrasions especially if associated with shock or unconsciousness. Again, use blood or plasma from similar type of injury if available

D. Surgery

1) Pre-surgery if operation is to be extensive, administer blood one hour before surgery and during and after surgery as indicated

2) Post-surgery

a. Following surgery where wound healing is slow

b. After extensive surgery and/or when operation time is over one hour. Consider using blood of individuals recovering from surgery if such blood or plasma is available. Administer at first daily, then every other day as for post burn--according to severity and need.

V. PUBLICATIONS DURING PERIOD OF REPORT

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